OPEN ACCESS
Advances in Animal and Veterinary Sciences

Research Article

The Effect of Black Cumin (*Nigella sativa*) on the *Caspase-3* Expression and the Number of Testic Sertoli Cells in *Balb/C* Male Mouses Exposed to Diazinon

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Abstract | Diazinon is an *organophosphate* pesticide that is often used in agricultural business sectors. Nevertheless, this type of pesticide could negatively inhibit the acetylcholinesterase, resulting cell apoptosis and hormonal disruption of the reproductive system. The major of this study was to evaluate the effectiveness of black cumin seeds as antioxidants on the expression of *caspase-3* and sertoli cells in spermatogenesis. A total of 20 male mouses were divided into five groups, K- (negative control, no treatment), K+ (positive control, 40 mg diazinon/kg BW); P1 (40 mg diazinon/kg BW + 200 mg black cumin/kg BW); P2 (40 mg diazinon/kg BW + 400 mg black cumin/kg BW); P3 (40 mg diazinon/kg BW + 600 mg black cumin/kg BW) throughout trials. The results showed that the black cumin seeds powder significantly decreased the *caspase-3* expression, whereby P3 revealed the lower value compared to other treatment groups ($P < 0.05$). Besides, an increased the number of Sertoli cells was significantly in line the supplementation of black cumin powder ($P < 0.05$), showing P3 had stronger effect than their counterpars. Furthermore, this study suggests that the administration of black cumin seed powder could decrease the expression of *caspase-3* and increase the number of Sertoli cells in mouses subjected diazinon.

Keywords | Black Cumin Seeds, Diazinon, Sertoli Cells, *Caspase-3*, Mouse.

Received | January 13, 2023; **Accepted** | February 20, 2023; **Published** | April 01, 2023
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Citation | Agustina GC, Syahfitri E, Hendrawan VF, Aditya S (2023). The effect of black cumin (*Nigella sativa*) on the *Caspase-3* expression and the number of testic sertoli cells in balb/c male mouses exposed to diazinon. Anim. Vet. Sci. 11(5): 791-794.
DOI | http://dx.doi.org/10.17582/journal.aavs/2023/11.5.791.794
ISSN (Online) | 2307-8316

INTRODUCTION

The utilization of pesticides is relatively high in agriculture sectors. According to BMC Public Health, about 720,000 cases of accidental pesticide poisoning, and approximately 44% of 840 million farmers worldwide experience pesticide poisoning throughout the year (Boedeker et al., 2020). Furthermore, diazinon is one of the pesticide frequently used due to its low cost. Nevertheless, this type of this pesticide could inhibit AChE in the blood, causing the disruption of blood circulation and neurotoxicity (Lesmana, 2013). Besides, about 4% of the cases of pesticide poisoning occurs in rice fields and dairy cows. Indeed, the contamination of pesticides in feed and food could cause residues in the body, subsequently hampering the health and reproduction aspects (Nining et al., 2019).

Furthermore, the consumption of antioxidant could enervate the harmful effect of diazinon when they entered the systemic circulation. Nowadays, there is high interest in developing therapeutic agents from various natural products to substitute the synthetic drugs due to their harmful effect (Danaei et al., 2018). Black cumin seeds are one of natural ingredients containing high antioxidant activity,

like *thymoquinone*. *Thymoquinone* is the major bioactive compounds acting as antioxidants and powerful anti-inflammatory agents. Besides, this compound could positively affect the nerve system of the brain by the secretion of the hormone FSH and inhibit apoptosis in the cells of the reproductive organs (Forouzanfar et al., 2014). Therefore, this study was conducted to examine the effect of black cumin seeds powder on the number of Sertoli cells and the expression of *caspase-3* in male mouse subjected diazinon.

MATERIALS AND METHODS

Animal and Experimental Design

The animal experiment was conducted at the Faculty of Veterinary Medicine, Brawjaya University (Malang-East Java, Indonesia). All procedures regarding the animal trial and treatments were approved by The Animal Ethics Committee of Brawijaya University (Malang-East Java, Indonesia). Besides, this experiment followed the animal research regulation of The Animal Welfare and Veterinary Public Health under The Republic of Indonesia (PP No.95/Tahun 2012/Kesehatan Masyarakat Veteriner dan Kesejahteraan Hewan).

A total of twenty *Balb/c* male mices (20-40 g; healthy condition; no inflammation) were selected for the experiment. The acclimation was done for 7 d prior to treatment. Furthermore, animals were divided into five different groups; K- (negative control, no treatment), K+ (positive control, 40 mg diazinon/kg BW); P1 (40 mg diazinon/kg BW + 200 mg black cumin/kg BW); P2 (40 mg diazinon/kg BW + 400 mg black cumin/kg BW); P3 (40 mg diazinon/kg BW + 600 mg black cumin/kg BW). The diazinon was orally submitted using gastric sonde daily for 8 wk. The mixture of feed and black cumin powder were given *ad libitum*. All the mices had an access to free water throughout the experiment.

Sampling, Preparation and Analyses

The edicular organs were collected at the end of experiment following previous protocols (Safithri, 2017). Furthermore, testicular immunohistochemical was done using the paraffin CPI procedures from The Cell Signaling Technology (Zulkarnain and Dicky, 2012). Meanwhile, the testicular histopathology was done using hematoxylin eosin staining (HE) following the prior procedures until further analyses and observation (Jusuf, 2009).

Statistical Analyses

The data was analyzed using the One Way Analysis of Variance (ANOVA) Test one-way pattern with a confidence level of 95% to find out the difference among groups. The significant difference among groups were considered with $P < 0.05$, meanwhile, $P > 0.05$ indicated no significant difference among groups.

RESULTS

Caspase-3 Testicles

The *caspase-3* expression on testicular immunohistochemical are shown in Figure 1. Meanwhile, the average area of *caspase-3* expression is pointed in Table 1. The data revealed that the supplementation of black cumin powder showing a decreased *caspase-3* expression in mices subjected diazinon ($P < 0.05$), whereby the declined the average area of *caspase-3* expression were more pronounced in P3 groups compared to their counterparts.

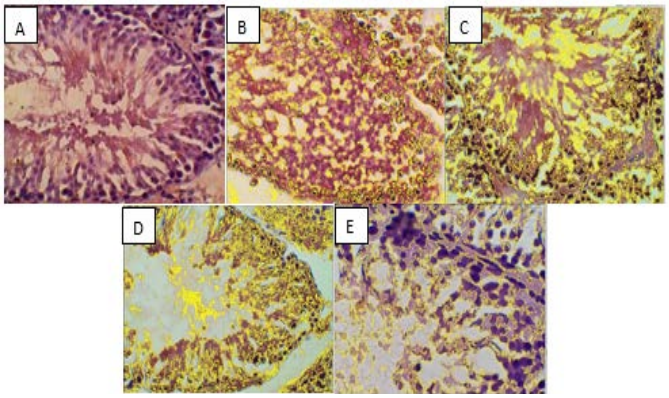

Figure 1: The expression of *caspase-3* in the testis mice negative control group (A), positive control (B), P1(C), P2 (D), and P3(E) in areas with yellow edge lines (👎) (400x).

Table 1: Expression of *caspase-3* on testicular immunohistochemical in mouses exposed to diazinon and treated with black cumin seed powder

Group	*Caspase-3* Expression (Area %) Mean ± SD
K-	0,75 ± 0.957[a]
K+	98,05 ± 1,088[e]
P1	73,05 ± 7,466[d]
P2	55,75 ± 4,795[c]
P3	36,2 ± 1,523[b]

Description: Different supercripts show a significant difference ($P < 0.05$).

Number of Sertoli Cells

Figure 2 shows The number of Sertoli cells of testicular in mice models. Meantime, the average number of Sertoli cells is summarized in Table 2. Overall, a significant difference was observed among the groups ($P < 0.05$). A more detailed comparison among groups showed the higher number of Sertoli cells was more pronounced in P3 groups compared to other treatment animals.

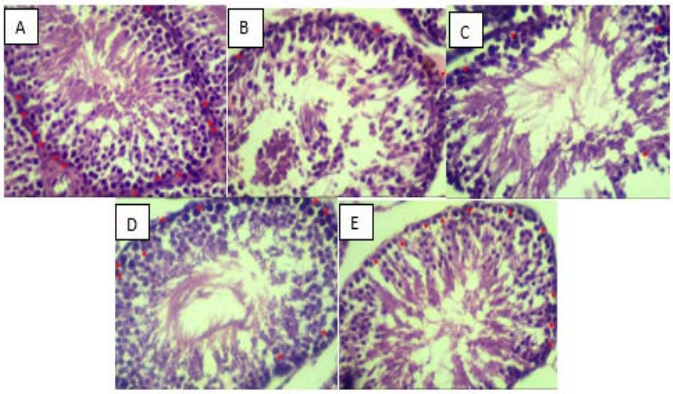

Figure 2: Sertoli cells on mice negative control (A), positive control (B), P1 (C), P2(D), and P3(E) with red arrow (▲) (400x)

Table 2: Average number of Sertoli cells in testes mices exposed to diazinon and treated with black cumin seed powder

Group	Sertoli Cell Count Mean ± SD
K-	21,15 ± 2,16[c]
K+	8,45 ± 0.957[a]
P1	12,35 ± 2,754[ab]
P2	17,45 ± 2,887[bc]
P3	18,9 ± 3,775[c]

Description: Different notations show a significant difference (P< 0.05).

DISCUSSION

The *caspase-3* expression in each treatment group showed differences in the brown area of expression due the reaction between DAB and the enzyme SA-HRP . This result indicated the presence of *caspase-3* expression in cells which act as a mediator or executor of apoptosis associated in the destruction of cells. Furthermore, the apoptosis of testicular cells could trigger a decreased the quality and production of spermatozoa and induce infertility (Sari, 2018). Interestingly, the supplementation of black cumin powder therapy showed a decreased the average of *caspase-3* expressions in mice exposed to diazinon. This result indicated the declined free radicals in the body, resulting the lower lipid peroxidation in endogenous membrane and *apopotosis* in testicular cells (Sari, 2018).

Besides, the decreased expression of *caspase-3* in treatment groups might be due to the therapy of black cumin powder as a medicinal plant with *thymoquinone* content that has antioxidant effects. Indeed, *thymoquinone* could impair the negative impact on free radicals by donating electrons to free radicals, and subsequently stabilizing them and stopping the chain of free radical reactions to the formation of excess ROS. These circumtances could trigger oxidative stress of cells and create the apoptosis episodes (Fajrin, 2013). It is known that antioxidants are able to stabilize unpaired electrons and control the excessive oxidative stress to prevent apoptosis (Munawaroh, 2011).

Furthermore, we found the lower number of Sertoli cells in the seminiferous tubule area of mices subjected diazinon. Indeed, the induction of diazinon-type *organophosphates* could inhibit AChE which subsequently induce the incoordination of nerve system. Besides, these circumstances cause the inhibition of the GnRH release, then negatively affect the secretion of FSH and Sertoli cells (Osiana and Mayasari, 2017). Interestingly, mice subjected diazinon and supplemented black cumin powder showing a higher number of Sertoli cells. This result indicates that the black cumin powder positively affects the regulation of FSH and Sertoli cells. More details, an increased number of Sertoli cells from all three treatments might be due to the black cumin powder containing *thymoquinone* which play role as an antioxidant compounds. Hereinafter, this compound donates its electrons to the ACh binding free radical affecting a decreased AChEI. By these conditions, the neural synapse could deliver the impulses to the hypothalamus of the brain, subsequently triggers an increased GnRH in the anterior pituitary for FSH and Sertoli cells production (Osiana and Mayasari, 2017).

CONCLUSION

The data revealed that the administration of black cumin powder in mices subjected diazinon could decrease the expression of *caspase-3* and increase the number of Sertoli cells. The data also pointed out that the black cumin powder with level 600 mg/kg BW show the strongest effects. Nevertheless, the further investigation concerning hormone profiles is suggested.

ACKNOWLEDGMENTS

The authors thank the Faculty of Veterinary Medicine, Brawijaya University-Indonesia for the financial support of this project (DPP/2020-Penelitian). The assistance of lab technician of Laboratory of Veterinary Reproduction-Brawijaya University is well appreciated.

CONFLICT OF INTEREST

The author declares there is no conflict of interest in publishing this article.

NOVELTY STATEMENT

This article has never been published and is not plagiarized from previous articles.

AUTHORS CONTRIBUTION

All authors contributed to the preparation of the article. The first author has the biggest contribution to compiling the article. A fourth author was also responsible for proof-reading. So the correspondence was also carried out by the first writer.

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